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Remarks

By this amendment, claim 33 is amended to specify that the non-neuronal cancer to be treated does not occur in the central nervous system. Support can be found throughout the specification, for example page 6 lines 13-22. No claims are added or cancelled. Therefore, claims 33 and 37-40 are still pending in the application. No new matter is added by this amendment.

Information Disclosure Statement

Enclosed are copies of the Information Disclosure Statement filed on June 2, 2003, and a copy of the reference cited therein. Applicants request that the Examiner acknowledge receipt and consideration of the Information Disclosure Statement and the one attached reference by returning a copy of the Form 1449 with the next action.

35 U.S.C. § 103(a)

Replication of HSV1716 in non-neuronal tissue

Claims 33 and 37-40 were rejected under 35 U.S.C. § 103(a) as obvious primarily in view of Martuza *et al.* (U.S. Patent No. 6,139,834) and Brown *et al.* (WO 92/13943), and in some examples, further in view of Roizman *et al.* (U.S. Patent No. 6,340,673). The rejection placed emphasis on the ability of HSV1716 to be capable of killing non-neuronal tumour cells via oncolysis "since it retains the ability to replicate in the peripheral tissues, yet it is non-neurovirulent, and safe" (for example see the emphasis at page 5 and page 7 of the Office action). Applicants disagree and request reconsideration.

HSV1716 is not considered to replicate effectively in peripheral tissue. There are important differences between peripheral **nervous** tissue and other peripheral tissues, and the replicative behaviour of HSV in those tissues. In the normal process of HSV infection, the HSV initially infects peripheral **nervous** tissues before axonal transport occurs resulting in an infection of the dorsal root ganglia followed by entry to the **central** nervous system. In many discussions of HSV, the literature makes the distinction between peripheral and central **nervous**

system tissues, the tissues normally infected by HSV. However, references are not made to HSV infection of non-nervous system tissues.

Although the Office action on page 5 refers to page 3 lines 7-11 of Brown *et al.* ("...strain 1716 . . . is incapable of replication in CNS neurons but is capable to elicit a good immunological and cell mediate response due to its ability to replication in the peripheral tissue."), this language from Brown *et al.* is referring to latent replication in peripheral nervous tissue following footpad inoculation (see page 13 line 30 to page 14 line 11 of Brown *et al.*).

To provide further evidence that HSV1716 replicates poorly in peripheral **non-nervous** system tissues, Applicants enclose a copy of Randazzo *et al.* (Virology 223:392-5, 1996; Exhibit A). This paper confirms that HSV1716 is a replication restricted virus (abstract). Several passages from Randazzo *et al.* are drawn to the attention of the Examiner:

> "We find that replication of 1716 is **severely** restricted in such human skin grafts relative to both parental wild-type HSV-1 strain 17+ and the HSV 1716 revertant virus" (abstract) (emphasis added).
>
> ICP34.5 "mutant viruses showed limited replication in the footpad, and replication in the eye could not be demonstrated following intraocular inoculation" (page 392 col 1).
>
> "... replication of HSV-1716 is severely restricted in human skin xenografts in vivo" (page 392 col 2).
>
> "The restricted nature of HSV1716 replication relative to that of wild-type 17+ in the human skin xenografts is further supported by gross and histologic examinations of the tissues at various times post infection" (page 393 col 2).
>
> "The majority of tissue sections of 1716-infected human skin xenografts examined did not show any histopathologic or immunohistochemical evidence of HSV infection" (page 393 col 2).

"The difference in titer of recovered virus between 1716 and wild-type 17+ seen in human skin xenografts is striking and parallels the restricted replication of ICP34.5 mutants seen in neuronal and nonneuronal tissues of rodents" (page 395 col 1).

In the final paragraph, the authors conclude that there is a "dramatic difference in replication between 1716 and 17+ in the human xenograft skin".

These results are also borne out in the present application (for example, see the example starting at page 13, line 16 of the specification), which indicates that HSV1716, in contrast to the wild-type (HSV-1 17+) does not replicate in the peripheral non-neuronal tissues, but only selectively in peripheral non-neuronal **tumor** tissue.

Therefore, the conclusions reached in the most recent Office action regarding the replication properties of HSV1716 are incorrect. In fact, HSV1716 was expected to exhibit very poor replication in peripheral non-nervous tissues. Other references are available in the state of the art that further indicate that HSV1716 was considered to replicate poorly in peripheral (i.e. non-nervous) tissue.

Safety and non-neurovirulence of HSV1716, whilst important for the utility of HSV1716 as a vaccine, are not factors relevant to the expectation of HSV1716 being able to replicate in non-nervous system peripheral tissues or being useful for the treatment of non-neuronal cancers.

Martuza *et al.* (US 6,139,834)

The skilled person in the art would not be motivated to carry out the method of claim 33.

The skilled person would have known that HSV1716 exhibited particularly poor replication properties in non-nervous system tissues. Therefore, if the skilled person had considered utilising HSV1716 in the method of Martuza *et al.* (as suggested on page 5 of the Office action) the skilled person would have been deterred from making the combination as he/she would have expected the poor replicative properties of HSV1716 to make HSV1716 ineffective in the lysis of the non-neuronal tumour cells.

The skilled person would also not have had a reasonable expectation of successfully treating non-neuronal cancer using HSV1716 due to the fact that he/she would not have expected replication (of the latent or lytic kind) to occur. Replication in the peripheral tissues is central to the ability of the HSV1716 to lyse the cells and treat the cancer, hence without the ability to replicate in the peripheral tissues, the skilled person would not have expected HSV1716 to be useful in the method of claim 33.

Roizman *et al.* (US 6,340,673)

Roizman *et al.* teaches nothing more about HSV1716 than Martuza *et al.*. The teaching in Roizman *et al.* "that infection of cells of neuronal origin with mutants incapable of expressing gamma 34.5 gene resulted in shutoff of cellular protein synthesis, whereas infection of cells of non-neuronal origin with wild type or mutant viruses resulted in sustained protein synthesis and production of infectious progeny" is relied upon in the Office action. However, the conclusion reached in the Office action is inconsistent with this statement.

The only cell types in which treatment of cancer is exemplified in Roizman *et al.* are neuronal cancer cell types. Reference to treatment of non-neuronal cancers is unsupported. As the only tumour types for which evidence of treatment is provided are neuronal, the indication that replicative mechanisms are different in cells of non-neuronal origin actually points to a different behaviour of the HSV mutants (R3616 and R4009) of Roizman *et al.* in these cells. However, because Roizman *et al.* does not investigate those differences, there is no teaching in that respect. Furthermore, the results would be specific to those mutants and could not be applied to HSV1716.

It is indicated on page 12 of the Office action that the replication defect in HSV1716 is only manifested in the central nervous system. The paper enclosed (Virology 223:392-5, 1996, Exhibit A) clearly shows this conclusion to be in error. Moreover, it is asserted in the Office action that the existence of a replication defect for HSV1716 only in the central nervous system allows the mutant HSV1716 to kill non-neuronal tumor cells via oncolysis. If this were true and HSV1716 showed replicative ability in all non-neuronal tissues it could not provide a useful

vaccine as it may indiscriminately replicate in all non-neuronal tissues, and could lead to lysis of tumor and non-tumor cells, which is an undesirable feature of any therapeutic vaccine. In fact, HSV1716 has been found by the Applicants to lyse only the non-neuronal tumor cells and not the surrounding healthy tissue and is therefore an excellent vaccine candidate.

Therefore in combining the teaching of Roizman *et al.* with the teaching of Brown *et al.*, the motivation and reasonable expectation of success are lacking. Further combining Martuza *et al.* with Roizman *et al.* and Brown *et al.* does not overcome the fact that the skilled person would have known that HSV1716 was "severely replication restricted" in peripheral non-nervous system tissues, and would therefore have lacked an ability to cause a lytic infection and have been expected to be useless in a method of treating a non-neuronal cancer by lysis of non-neuronal tumour cells.

Ribonucleotide reductase mutation in Martuza *et al.* is not merely a Preferred Embodiment

It is indicated on page 4 of the Office action that the inclusion of a ribonucleotide reductase mutation in Martuza *et al.* is only in a preferred embodiment. Applicants disagree. This feature appears in independent claim 1 and in the summary of invention where it is a feature of an aspect of the invention which satisfies the objects of the applicant (see col 3 lines 43-48). The description also indicates that "Viruses of the instant invention are engineered to contain alterations in the expression of at least two specific HSV-1 genes: (1) the $\gamma\gamma$34.5 gene **and** (2) the ribonucleotide reductase gene." (col 5 lines 35-38) (emphasis added).

Considering the examples, of which only examples 1-3 have actually been performed (examples 4-8 are prophetic, and in any event refer to viruses "of the invention", i.e. having both mutations), all of examples 1-3 are concerned with construction and testing of the **double** mutant G207. Furthermore, testing is only performed on neuronal cell types (such as the glioma cell line U-87MG (col 16 line 19) and glioma xenografts (example 3)).

Therefore, to conclude that the ribonucleotide reductase mutation is only a "preferred embodiment" (page 4 of the Office action) is in error. For the foregoing reasons, the

ribonucloetide reductase is clearly an essential feature of the invention and all of its embodiments.

In view of this conclusion, Martuza *et al.* is even further from Applicants' invention than indicated in the Office action, because Martuza *et al.* requires both a γ34.5 mutation **and** a ribonucleotide reductase mutation, the contribution and effect of each mutation being unclear and the essential nature of the latter mutation being indicative that it is required for tumor cell killing.

It is also noted in the Office action that Roizman *et al.* mentions treatment of tumorogenic diseases both in the CNS and in all other parts of the body (col 5 lines 63-66). However, **all of the examples** in Roizman *et al.* relate to testing of neuronal cells (such as neuroblastoma cell lines). There is no teaching in Roizman *et al.* that informs the skilled person whether or not the Roizman *et al.* mutants (R3616 and R4009) are actually useful in treating non-neuronal tumors.

In contrast to the prior art, the claims of the present invention relate to the specific virus HSV1716, which is a variant of HSV-1 strain 17+. The viruses constructed in Martuza *et al.* are derived from the HSV-1 strains KOS and F (col 13 line 17) and the viruses constructed in Roizman *et al.* derived from strain F. The properties of the strain 17+ variant HSV1716 are not predictable from those of other strains, and in particular, the properties of HSV1716 in non-neuronal cells are not predictable from the results of experiments with strain F or KOS variants which have only been performed in neuronal cells.

In summary, Applicants' invention is concerned with the treatment of non-neuronal tumors by injection of the specific virus HSV1716. HSV1716 replication is "severely restricted" in non-neuronal tissues. The skilled person would have considered that the methods described in Martuza *et al.* and Roizman *et al.* could not have been applied to HSV1716 with any expectation of success because replication causing lysis in non-neuronal tumors is needed for success of the method. As a result, the skilled person would also have lacked motivation to try such a method. In contrast to this teaching, the Applicants have shown that HSV1716 can in fact be used for the

treatment of non-neuronal tumor cells by lysis. This result is surprising and cannot be considered obvious under terms of 35 U.S.C. § 103(a).

Based on the foregoing arguments, and the amendments presented herewith, Applicants request that the rejections of the claims under 35 U.S.C. §103(a) be withdrawn.

Double patenting Rejection

Copending U.S. Patent Application No. 08/776,350 presently claims:

> A method of treating a metastatic tumour which occurs in but does not originate from the central nervous system of a human, which method comprises the step of administering to said human an effective amount of an avirulent herpes simplex virus type I having a non-functional γ34.5 gene, wherein the HSV-1 infects and replicates within the tumour cells.

In order to avoid the possibility of double patenting, Applicants have amended claim 33 to clarify that the cancer does not occur in the central nervous system. This amendment avoids any possibility that the claims of the present application could read onto those of the copending U.S. Application No. 08/776,350, as the claims of the latter application refer to a tumor that must occur in the central nervous system and the amended claim submitted herewith excludes this possibility. The claims of the two applications are now patentably distinct.

Applicant respectfully submits that the provisional obviousness-type non-statutory double patenting rejection has been overcome, and requests that it be withdrawn.

Conclusion

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending claims in this application is believed warranted. The Examiner is invited and encouraged to telephone the undersigned to enhance the efficient prosecution of this application to issue.

Respectfully submitted,

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SHORT COMMUNICATION

Herpes Simplex 1716—an ICP 34.5 Mutant—Is Severely Replication Restricted in Human Skin Xenografts *in Vivo*

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HSV-1716 is a replication-restricted, neuroattenuated ICP 34.5 gene mutant of herpes simplex virus type 1 (HSV-1). Because of the attenuated phenotype of ICP 34.5 mutants in rodent models of HSV disease, they have been promoted as potential vaccine strains and gene therapy vectors and have been used by us and others as therapeutic agents for the treatment of experimental malignant tumors. However, all data on the phenotype of HSV-1716 and other ICP 34.5 mutants are from animal model systems, while humans are the natural hosts of HSV-1. To achieve an initial characterization of the phenotype of 1716 in human tissue, we have studied its replication in mature human skin xenografts on SCID mice. We find that replication of 1716 is severely restricted in such human skin grafts relative to both parental wild-type HSV-1 strain 17^+ and the HSV-1716 revertant virus 1716R, in which the 759-bp ICP 34.5 gene deletions have been repaired. Moreover, the replication of both 1716 and 17^+ is significantly better in the human skin grafts than it is in mouse skin. The implications of these findings are discussed.

HSV-1716 has a 759-bp deletion in both copies of the ICP 34.5 gene (*1*). Deletion or mutation of the ICP 34.5 gene results in HSV-1 variants that are incapable of replicating in the central nervous system of mice and do not cause encephalitis when inoculated via various routes (*1–3*). This is in sharp contrast to wild-type HSV-1, which grows exponentially in brain and kills mice within days of inoculation (*1*).

In vitro, ICP 34.5 HSV-1 mutants grow as well as wild-type virus on dividing cells of most established cell lines (*1, 2, 4*). However, on nondividing cells such as confluent primary mouse embryo cells, these mutants show impaired replication (*4*). In previous studies, we, and others, have examined the replicative phenotype of ICP 34.5 HSV-1 mutants *in vivo* in nonneuronal tissues in mice. These mutant viruses showed limited replication in the footpad (*5*), and replication in the eye could not be demonstrated following intraocular inoculation (*6, 7*).

We have recently shown that 1716 induced regression of preformed experimental intracranial melanoma and significantly improved survival times of treated tumor bearing mice (*8*). Similar results with other types of brain tumors have been demonstrated by others using comparable HSV-1 ICP 34.5 mutants (*9, 10*). The exciting potential of HSV-1 ICP 34.5 mutants as vaccine strains, and therapeutic agents for treatment of brain tumors, has prompted us to explore the *in vivo* phenotype of 1716 in more detail. Human skin xenograft systems have been employed to achieve replication of varicella zoster virus, human papilloma virus, and molluscum contagiosum virus, which are fastidious human dermatotrophic viruses (*11–13*). In the case of HSV-1, the use of a human skin xenograft system allows for studies in a prototypic human tissue of natural acute infection.

We find that the replication of both HSV-1716 and wild-type HSV-17^+ is much more efficient in the human skin than it is in mouse skin. Moreover, we find that relative to wild-type HSV-17^+, and the revertant virus HSV-1716R, the replication of HSV-1716 is severely restricted in human skin xenografts *in vivo*.

The titration data shown in Fig. 1 demonstrate the restricted nature of HSV-1716 in the human skin xenografts relative to wild-type HSV-17^+ and the revertant virus HSV-1716R. HSV 1716 shows only a slow increase in titer over the 3 days examined. In contrast, HSV-17^+ demonstrates exponential growth in the human skin over the same time period. To confirm that the phenotype of 1716 in the human skin was attributable to deletion of the ICP 34.5 gene, and not an additional unknown mutation, we also examined the replication of a revertant virus—1716R—in which the 759-bp ICP 34.5 gene deletions have been repaired (*14*). As shown in Fig. 1, 1716R demonstrated brisk replication in the human skin grafts and thus has a phenotype like that of wild-type 17^+.

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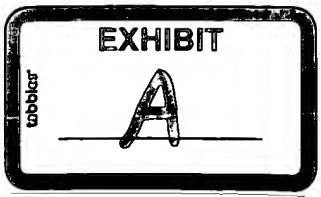

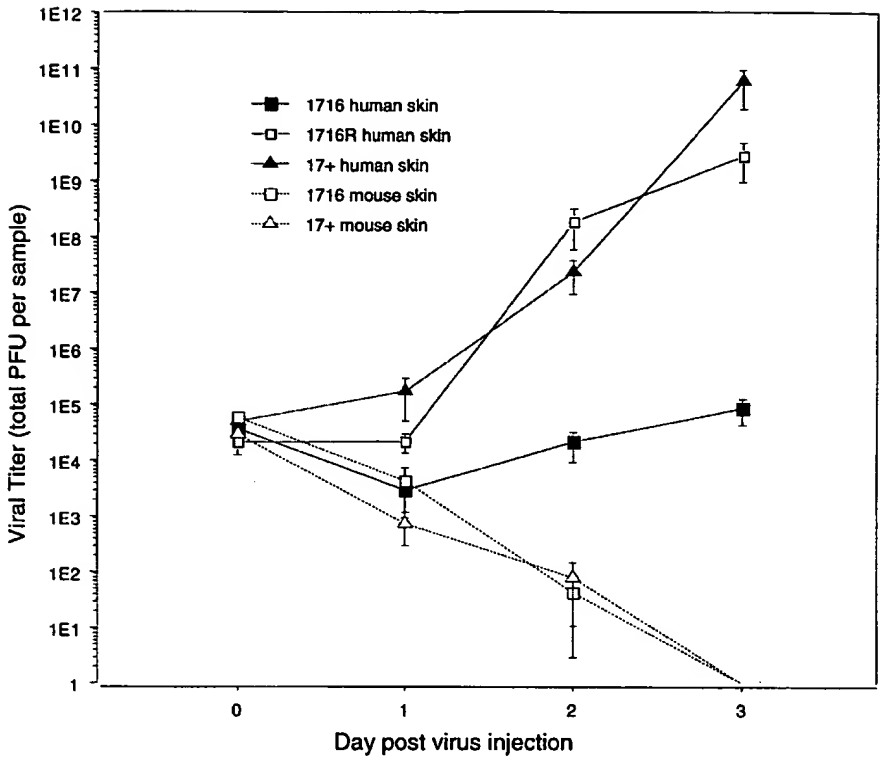

FIG. 1. Replication of HSV 17, 1716R, and 1716 in mouse skin and mature human skin xenografts. Full-thickness human foreskin xenografts were produced on SCID mice as previously described (*17*). Defatted full-thickness human skin grafts, ~1.5 cm in diameter, consisting of neonatal foreskin from elective circumcision, were grafted onto the flank of mice after removing full-thickness skin at the recipient site down to fascia. The grafts were sewn on, covered with an adhesive bandage clipped to the mouse, and covered with cloth tape. The grafts were left undisturbed until they had matured for 4–6 weeks. For inoculation directly into mouse skin, an ~5-cm^2-area patch of hair was removed from one flank using a chemical depilatory agent 1 day prior to viral inoculation (Magic Shaving Powder, Carson Products Co., Savannah GA). Mice were anesthetized with im ketamine/xylazine, and 5 × 10^6 PFU of HSV 17^+, 1716R, or 1716, in a total volume of 50 μl, were injected intracutaneously into either their flank skin or within mature human skin xenografts using a Hamilton syringe and a disposable 30-gauge needle. At the various times shown, mice were sacrificed by lethal injection of anesthesia, and the entire xenograft, or the ~5-cm^2 patch of mouse flank skin surrounding the site of inoculation, was removed aseptically to the level of fascia and frozen in liquid nitrogen. At the time of viral titration assay, each tissue sample was rapidly thawed in a 37° water bath, and the tissue was homogenized in viral culture medium at a 10% weight/volume ratio using a Pyrex Ten Broeck tissue grinder. The homogenates were centrifuged at 3000 g for 10 min at 4°. The supernatant of each tissue homogenate was diluted logarithmically in media, and the viral titer of each was determined in triplicate by plaque assay on BHK cells (*18*). Each data point represents the mean ± standard error of two tissue samples, and the experiment shown was repeated with similar results.

Comparison of the replication of 1716 and 17^+ virus in the human skin xenografts to that in SCID mouse skin demonstrates that both viruses replicate much better in the human skin. As shown in Fig. 1 intracutaneous inoculation of SCID mouse flank skin resulted in a level of replication of both 1716 and 17^+ that was just at the threshold of our titration assay. Despite a continual drop in titer over the 3 days examined, we believe that the rather slow decline most likely represents low-level replication rather than a complete absence of replication.

The restricted nature of HSV-1716 replication relative to that of wild-type 17^+ in the human skin xenografts is further supported by gross and histologic examinations of the tissues at various times postinfection. Hematoxylin and eosin sections are paired with sections stained immunohistochemically for HSV-1 antigens. HSV cytopathic effect and antigen staining within the human skin xenografts subsequent to HSV-1716 infection were scant, focal, and restricted to the epidermis at all times examined. In fact, the sections shown in Fig. 2 for 1716 were selected specifically to show evidence of 1716-induced pathology. The majority of tissue sections of 1716-infected human skin xenografts examined did not show any histopathologic or immunohistochemical evidence of HSV infection.

In contrast to HSV-1716, wild-type 17^+ showed gross, histopathologic, and immunohistochemical evidence of robust replication and pathologic effects. By Day 3 postinoculation focal epidermal histopathologic and immunohistochemical effects attributable to HSV were clearly seen. By Day 7 postinoculation with 17^+, near total destruction of the human skin grafts was seen. As is evident in the representative gross photograph in Fig. 2, full thickness epidermal ulceration is present in the majority of the xenograft. The representative histologic section from Day 7 post 17^+ infection shows complete loss of the epidermis with necrotic crust present. Immunohistochemical evaluation shows HSV antigen staining at the

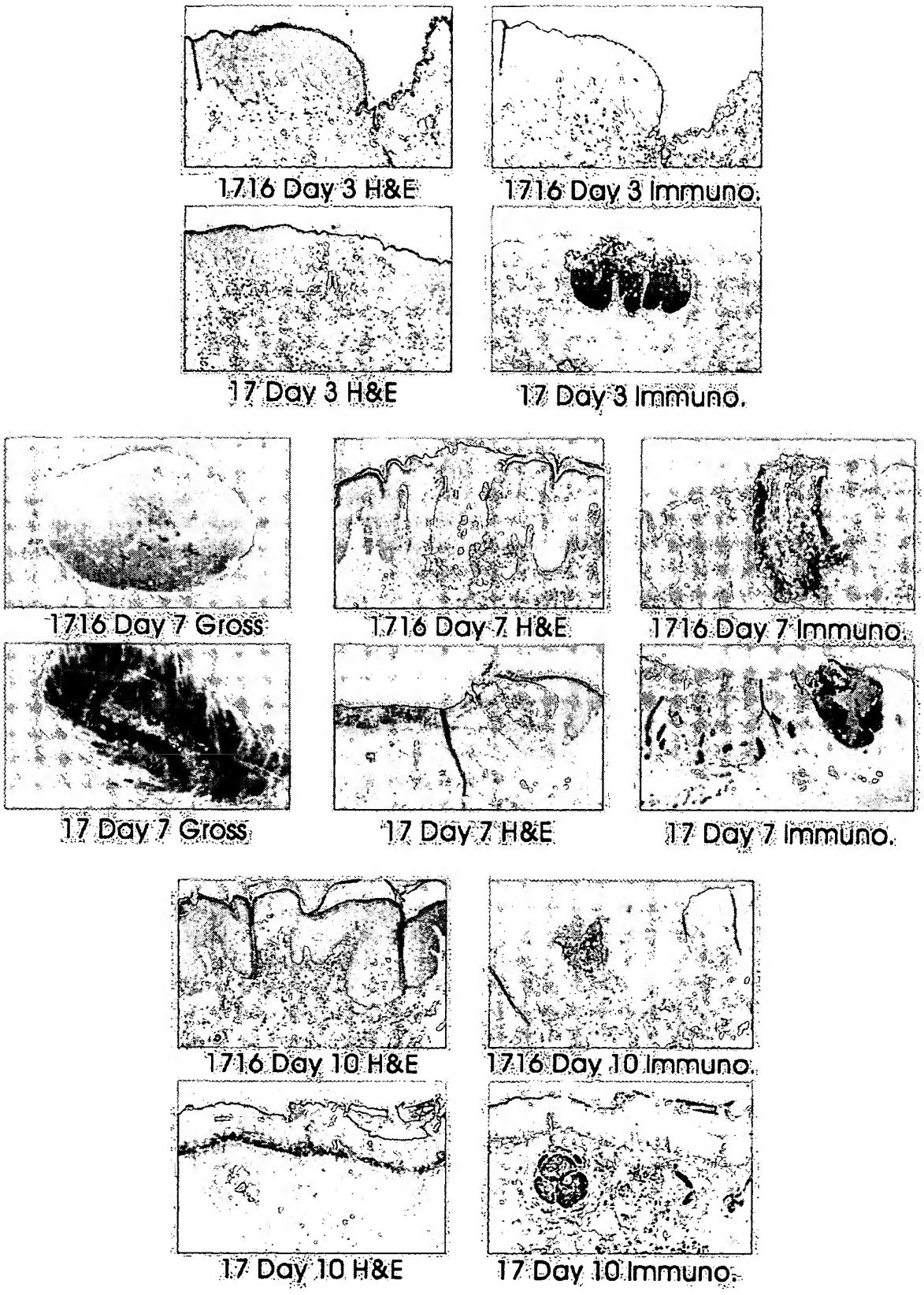

FIG. 2. Comparison of the gross and histologic changes caused by HSV-17 and 1716 in mature human skin xenografts. Full-thickness human foreskin xenografts were produced on SCID mice as described in the legend to Fig. 1. Mice were anesthetized with im ketamine/xylazine, and 5×10^6 PFU of HSV 17^+ or 1716, in a total volume of 50 μl, were injected intracutaneously within mature human skin xenografts using a Hamilton syringe and a disposable 30-gauge needle. At the various times shown, mice were sacrificed by lethal injection of anesthesia, and the entire xenograft was removed aseptically to the level of fascia and fixed in 10% neutral buffered Formalin. Histologic sections for light microscopy were stained with hematoxylin and eosin. Parallel sections were stained for HSV antigen expression using an indirect avidin–biotin immunoperoxidase method (Vector Labs, Burlingam, CA) as specified by the manufacturer with slight modifications developed in our laboratory (*19*). Rabbit antiserum to HSV-1 (Dako Corp., Carpinteria, CA) was used at a dilution of 1:1000. Sections were counterstained with hematoxylin. As a control for the specificity of immunostaining, nonimmune rabbit serum was substituted for the primary HSV-1 antiserum (data not shown). Magnification is 200× in all histologic sections.

edge of the xenograft and staining of adnexal structures within the dermis. By Day 10 post 17^+ infection, all sections examined exhibited complete epidermal destruction and significant dermal adnexal infection by immunohistochemistry.

When HSV-1716 or HSV-17^+ was inoculated into depilitated mouse skin, no gross alteration was seen surrounding the site (data not shown).

This study provides initial information on the phenotype of the HSV-1 ICP 34.5 mutant 1716 in human tissue. Although the restricted nature of the replication of this virus in neuronal tissues is well established (*1, 2, 4*), the replication of this and other ICP 34.5 mutants in nonneuronal tissues is less well documented. A study examining the replication of 1716 in mouse footpad found that the peak viral titer on Day 1 postinfection was comparable to that of wild type, but that the duration of recovery of titerable virus was several days shorter for the mutant (*5*). The peak vaginal viral titer of an HSV-1 strain F ICP 34.5 mutant was shown to be approximately 100-fold less than that of wild type on average over Days 1 to 8 post intravaginal infection in mice and guinea pigs (*7*). No replication of two ICP 34.5 mutants engineered from different parental strains was detectable in the eyes of mice infected via scarified cornea (*6, 7*). The difference in titer of recovered virus between 1716 and wild-type 17^+ seen in human skin xenografts is striking and parallels the restricted replication of ICP 34.5 mutants seen in neuronal and nonneuronal tissues of rodents. Immunohistochemical evaluation of infected human skin xenografts for HSV-1 antigen demonstrates that antigen staining is focal in nature and occurred only in the epidermis. The polyclonal antibody used for immunohistochemistry was raised to an infected cell lysate and detects both structural and nonstructural HSV antigens. The titration data document production of progeny HSV-1716. Thus, we feel that the focally limited antigen staining observed is best interpreted as quantitatively limited, but qualitatively typical, viral replication rather than an abortive infection. It has been shown that replication of 1716 in stationary state mouse 3T6 cells *in vitro* is severely restricted and that a defect in virus maturation and egress from the nuclei of infected cells occurs (*15*). It is possible that this same defect occurs in the human skin xenografts and accounts for our findings.

A somewhat unexpected finding of this study was the inability to detect brisk replication of wild-type 17^+ replication in SCID mouse flank skin following intracutaneous infection. We and others have shown replication of 17^+ and other wild-type HSV-1 isolates in peripheral tissues of rodents (*5–7, 16*). Generally, significant trauma to the tissue such as preinjection of the footpad with hypertonic saline, abrasion of the footpad or lip with an emery board, scarification of the cornea with a needle, or abrasion of the vaginal mucosa with a cotton applicator stick is necessary to successfully inoculate virus at peripheral sites (*6, 7, 16*). For the purpose of this study a relatively nontraumatic intracutaneous injection was used. Although this is clearly sufficient to establish an ongoing infection in the human skin xenografts, it is apparently not sufficient to do so in murine flank skin in the case of 17^+. We are presently investigating the phenotype of other "wild-type" clinical isolates and mutants in human skin xenografts.

While the dramatic difference in replication between 1716 and 17^+ in the human xenograft skin offers encouragement as to the attenuated nature of ICP 34.5 HSV-1 mutants in nonneuronal human tissues, the impressive differences in replication found between the human and mouse skin for both mutant and wild-type HSV offer yet another reminder that the mouse is a far from perfect model for study of a human pathogen such as HSV-1.

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